

**DEVELOPMENT OF SYNGENEIC MOUSE MODELS TO STUDY THE THERAPEUTIC EFFICACY OF CHIMERIC ANTIGEN RECEPTOR T CELLS AGAINST SOLID TUMORS**

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**Background** Chimeric antigen receptor (CAR)-T therapy has made significant progress in treating hematological malignancies. However, its clinical benefits for patients with solid tumors have been unsatisfactory. The ineffectiveness of CAR-T therapy in solid tumors is believed to be attributed to the tumor microenvironment (TME). Unfortunately, replicating the TME in xenograft models is challenging due to the absence of native immune stroma. Therefore, the commonly used xenograft models lack the essential components to study human CAR-T effectively. To address this limitation, we have developed syngeneic mouse models that allow for the investigation of CAR-T efficacy in the immunocompetent setting.

**Methods** We developed two murine CAR-T models – one that targets glypican 3 (GPC3) and another that targets claudin 18.2 (CLDN18.2), as representative CAR tumor-associated antigens (TAAs). Anti-GPC3 and anti-CLDN18.2 CAR-T cells were tested both in vitro and in vivo against 4T1 cells overexpressing GPC3 (4T1-GPC3) and CT26 cells overexpressing CLDN18.2 (CT26-CLDN18.2), respectively.

**Results** CAR-T cells generated by retroviral transduction demonstrated efficient and antigen-specific cytotoxicity towards tumor cells in vitro. This cytotoxicity was accompanied by IFN $\gamma$  release and the upregulation of activation markers. Total body irradiation (TBI) was used to induce lymphodepletion in tumor-bearing mice, a process recapitulating the short course of chemotherapy cancer patients receive prior to CAR-T infusion. The dosage of TBI was determined to achieve sustained leukopenia while minimally impacting tumor growth. We observed substantial tumor control and extended survival in mice treated with either fresh or cryopreserved CAR-T cells compared to those infused with untransduced T cells. Notably, no treatment-related toxicity was observed with any of the CAR-T doses used in our studies.

**Conclusions** We have established two CAR-T mouse models in the immunocompetent setting. The first model, 4T1, represents a ‘cold tumor’ characterized by low immune infiltrates. The second model, CT26, represents a ‘hot tumor’ with high immune infiltrates. These models not only enable us to investigate the behavior of CAR-T cells within a more authentic TME, but they also serve as a valuable platform for evaluating the efficacy of our novel CAR-T products in a setting that more closely resembles cancer patients.

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